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Determining the Optimal Substrate for the Invasive New Zealand Mud Snail (*Potamopyrgus antipodarum*)

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ABSTRACT

The New Zealand mud snail (*Potamopyrgus antipodarum*) is an aquatic invasive species that has been found in many countries and six continents. *P. antipodarum* can reproduce at a high rate, which leads to a high population growth rate and successful spread of the snail. This could negatively impact ecosystems. The purpose of this work was to determine if certain habitats in streams are better for the mud snail than others. In this experiment, snails were taken from a population in Spring Creek in Centre County, PA. The snails were grown for eight weeks on four different diets, including leaf litter, woody debris, rocks, and *Spirulina* algae powder, which was the control. The size of each snail was measured at the beginning and end of the experiment to determine the mean growth rate. There was a significant difference in growth rates between the different treatments. Snails that grew on leaf litter and woody debris showed higher snail growth rates in comparison to the control and rock treatments. This suggests that some diets are better than others for the mud snail, and that the energy input from outside the aquatic system may be very important in the success of this invader.

TABLE OF CONTENTS

LIST OF FIGURES iii

LIST OF TABLES iv

ACKNOWLEDGEMENTS v

Chapter 1 Literature Review 1

Chapter 2 Introduction 3

Chapter 3 Methods 6

- Experimental Preparation 6
- Experiment 7
- Experiment Maintenance 7
- Data Collection 8

Chapter 4 Results 9

Chapter 5 Discussion 11

Chapter 6 Conclusion 14

Chapter 7 Appendix 15

BIBLIOGRAPHY 19

LIST OF FIGURES

Figure 1 The effect of the substrate type on the mean growth rate. 9

LIST OF TABLES

Table 1 P values from the pairwise contrasts of each treatment using univariate analysis of variance. 10

Table 2 Results from a univariate analysis of variance comparing log transformed data of all treatments. 15

Table 3 Results from a univariate analysis of variance comparing log transformed data of the control treatment to the leaf treatment. 15

Table 4 Results from a univariate analysis of variance comparing log transformed data of the control treatment to the rock treatment. 16

Table 5 Results from a univariate analysis of variance comparing log transformed data of the control treatment to the wood treatment. 16

Table 6 Results from a univariate analysis of variance comparing log transformed data of the leaf treatment to the rock treatment. 17

Table 7 Results from a univariate analysis of variance comparing log transformed data of the leaf treatment to the wood treatment. 17

Table 8 Results from a univariate analysis of variance comparing log transformed data of the rock treatment to the wood treatment. 18

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Chapter 1

Literature Review

Biological invaders can be a threat to ecosystems as they could alter different properties of habitats, affect biodiversity, and lead to biotic homogenization (Enserink, 1999) (Kolar & Lodge, 2001) (Cambray, 2003) (Mills, et al., 2003) (Duft, et al., 2003a) (Duft, et al., 2003b) (Alonso, 2005). One of the newer species that causes problems in the United States is the aquatic invasive New Zealand mud snail (*Potamopyrgus antipodarum*). There are two groups of populations of the mud snail in North America (Bilka & Levri, 2013). The first population was found in the 1980s in the western United States (Zaranko, et al., 1997) (Proctor, et al., 2007). The second population was found in the Laurentian Great Lakes (Zaranko, et al., 1997) (Proctor, et al., 2007).

P. antipodarum is known for its high capacity of reproduction (Geist, et al., 2022). This increases the population growth rate as well as the spread of the snail (Geist, et al., 2022). One adult individual mud snail can produce about 230 juveniles every year (Møller, et al., 1994) (Richards, 2002). So far, the snail has been found in at least 40 countries and six continents (Taybi, et al., 2021) (Geist, et al., 2022).

Diet can affect the invasion success of *P. antipodarum* (Bilka & Levri, 2013). Previous studies showed that the mud snail consumes periphyton, macrophytes, and detritus (Dorgelo & Leonards, 2001) (Jensen, et al., 2001) (Alonso & Camargo, 2003). Research on physical habitat preferences of *P. antipodarum* in invaded ecosystems is still limited (Geist, et al., 2022). Hence,

this study aims to determine the effect of the different substrates, where food grows, on influencing the individual growth rates of the invasive New Zealand mud snail.

Chapter 2

Introduction

The New Zealand mud snail is an aquatic invasive species. It has been found in various rivers, lakes, and estuaries in at least 40 countries and six different continents (Taybi, et al., 2021; Geist, et al., 2022). *P. antipodarum* can perform sexual and asexual reproduction in its native range. However, in invasive populations, *P. antipodarum* is parthenogenetic, meaning it is asexual and consisting only of females (Lively, 1987) (Jokela, et al., 1997) (Gangloff, 1998) (Jensen, et al., 2001) (Duft, et al., 2003a) (Duft, et al., 2003b). The mud snail reaches sexual maturity at a shell length between 3.0 mm and 3.5 mm (Møller, et al., 1994) (Richards, 2002). An adult individual could produce approximately 230 juveniles per year (Møller, et al., 1994) (Richards, 2002).

Due to the mud snail's ability to reproduce at a high capacity, leading to a high population growth rate, the mud snail could rapidly spread and consume the primary production of the ecosystem at a high rate (Geist, et al., 2022). Accordingly, the successful spread of the snail could be very harmful in ecosystems. Biological invasions could also alter certain properties of habitat, which could ultimately decrease biodiversity and lead to biotic homogenization (Enserink, 1999) (Kolar & Lodge, 2001) (Cambray, 2003) (Mills, et al., 2003) (Duft, et al., 2003a) (Duft, et al., 2003b) (Alonso, 2005).

The spread of *P. antipodarum* is aided by the fact that it can attach to waterfowl and has the ability to survive in the digestive tracts of fish and other species (Geist, et al., 2022). *P. antipodarum* could also influence carbon and nitrogen cycles in the ecosystems it invades (Hall, et al., 2003) (Alonso & Castro-Díez, 2012). Another study showed that *P. antipodarum* forces

native species to consumer lower quality food because it prefers consuming high nutrient quality algae (Riley, et al., 2008).

Several studies have shown that the mud snail's diet includes periphyton, macrophytes, as well as detritus (Dorgelo & Leonards, 2001) (Jensen, et al., 2001) (Alonso & Camargo, 2003). Yet, there has been limited studies performed on the physical habitat preferences of *P. antipodarum* in its invaded regions (Geist, et al., 2022). Therefore, this study focuses on determining the effect of the type of substrate where the snail consumes its food on the individual growth rates of the invasive New Zealand mud snail.

Periphyton is created when algae attach to different substrates (Kalff, 2002). There are different types of periphyton, including epiphytic, epibenthic, epilithic, and epipelic periphyton (Kalff, 2002). Epiphytic periphyton is algae grown on other types of plants (Kalff, 2002). Epibenthic periphyton is a term that is used to describe algae grown on green or brown stones or wood (Kalff, 2002). Epilithic periphyton is algae grown specifically on stones, whereas epipelic periphyton is algae grown specifically on wood sediments (Kalff, 2002). Periphyton with its associated community of bacteria, fungi, protozoans, and metazoans create what is known as a biofilm (Kalff, 2002).

For this experiment, three different substrates were used, including the leaf, rock, and wood substrates. Each substrate cultivates a different type of biofilm community (Kalff, 2002). Some biofilms may be better diets than others for the snail, which could affect snail feeding preferences, thus affecting the snail's growth rate (Kalff, 2002).

The purpose of this experiment is to determine if some substrates lead to higher growth rates in the mud snail than others. It was hypothesized that the leaf, rock, and wood treatments would all lead to higher growth rates in the mud snail in comparison to the control treatment. The

leaf treatment was hypothesized to show the highest mean growth rate because it was thought that it might be easiest for the snail to grow and feed on it due to the ability of leaf litter to decompose faster than wood.

Chapter 3

Methods

Experimental Preparation

Snails were collected on May 28th, 2021, from Spring Creek, Centre County, PA at 40.8507; -77.8224. Fishing waders and strainers were used to collect 100 snails. The snails were placed in 1 liter plastic containers filled with stream water. The snails were separated into four different containers to provide them with more space and lower the population density per container. The snails were fed *Spirulina* algae powder.

To provide periphyton covered rocks for the experiment, rocks and water from Spring Run on the Penn State Altoona campus were added to two containers. The pH of the water from the Spring Run collection site was measured and was around 7.6. The rocks were maintained by adding water to plastic containers and keeping the rocks in the same orientation where they always remained in the same side up. The rock containers were placed on a windowsill to ensure exposure to sunlight to allow for periphyton growth. The rock containers were also randomly rotated to avoid bias. Wood and Elm leaf litter were collected from Spring Run. They were rinsed to remove any animals from them. The wood was cut into small disc-shaped pieces using a saw. The diameter of the wood disc-shaped pieces was measured using a caliper. The average diameter was 28.5 mm. The disc-shaped wood pieces and the Elm leaf litter were placed into two separate buckets and water from the stream was added. The diameter of the rocks and the sizes of the leaves were also measured using calipers. The average diameter of the rocks was 31.3 mm and the average size of the leaves was 28.2 mm. A total of 100 juvenile snails, which were

between 0.5 mm and 1.0 mm in length, were collected from the SC 009 population from Spring Creek, Centre County, PA, and were placed in new containers to allow for periphyton growth.

Experiment

The main experiment began on September 24th, 2021. Using tape and a Sharpie, 200 mL plastic cups were labeled with the initial snail sizes. The snail sizes were measured to the nearest 0.1 mm using an ocular micrometer in a dissecting microscope. There were four different treatments. Treatment 1 had rocks. The rocks were added to the small cups in the same orientation that they were initially in when they were in the big container. Treatment 2 had Elm leaf litter, which were cut to ensure they were about the same size. Treatment 3 had disc-shaped wooden pieces. Treatment 4 had the standard lab diet of *Spirulina* algae powder and was considered the controlled treatment. There was a total of 25 snails per treatment, where each snail was placed in a separate cup, and where three quarters of each cup was filled with stream water. Each treatment was placed on a separate tray near the window to allow for exposure to sunlight. An analysis of variance was used to make sure that the mean initial size of the snail was not different between treatments.

Experiment Maintenance

The experiment was maintained three times per week. Stream water was added to ensure that the cups were always three quarters full to compensate for the evaporated water. Three drops of *Spirulina* algae powder were added to each cup of the control treatment three times per week. Oxygen was also provided using aeration for five seconds per cup three times per week. The

trays for each treatment were rotated three times per week to allow for random and equal light exposure. The experiment was maintained for eight weeks.

Data Collection

The experiment was completed on November 15th, 2021. Each snail was measured again using an ocular micrometer in a dissecting microscope. The growth rate of each snail was calculated as number of millimeters growth per day. This data was log transformed to meet the homogeneity of variances assumption of the statistical test. The raw data was collected in Microsoft Excel and observations were recorded. The SPSS statistical software was used to create univariate analyses of variance and tests for the effects of treatment on the log of growth rate. A Levene's test of equality of error variances was also performed to be sure that the data met the homogeneity of variances assumption of the statistical test. The variances between all the treatments were not significantly different ($p > 0.15$ in all cases).

Chapter 4

Results

In a univariate analysis of variance using all treatments, a statistically significant effect of treatment was found, indicating that the food source influenced the growth rate ($F = 37.25$, $df = 3$, $p < 0.001$) (Figure 1).

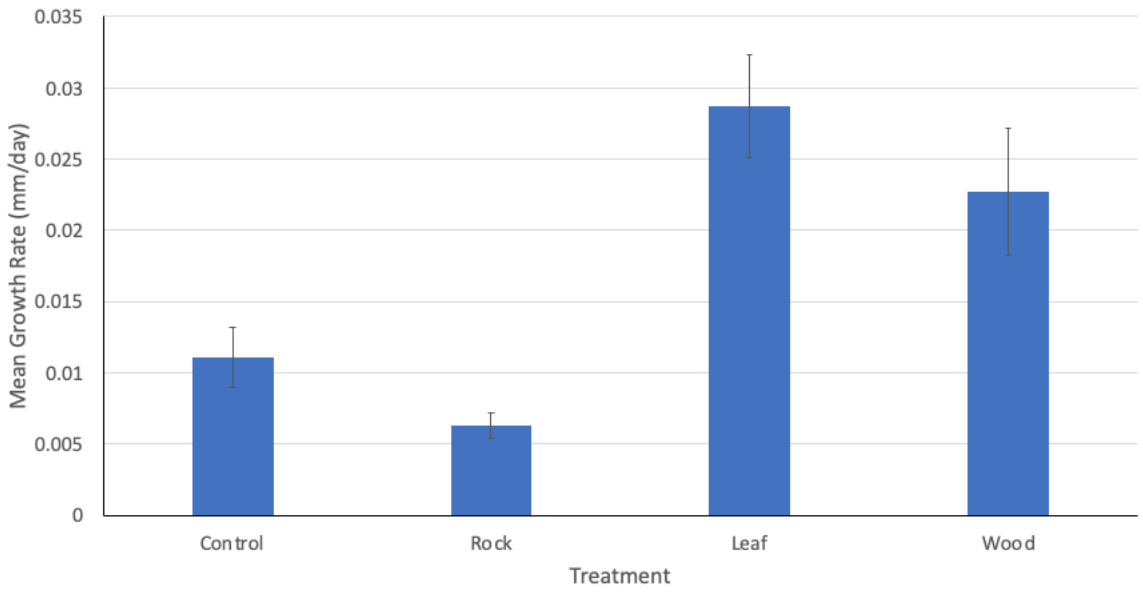

Figure 1 The effect of the substrate type on the mean growth rate in millimeters per day. The error bars are standard errors.

Table 1 below shows the results of individual pairwise comparisons between each treatment using univariate analysis of variance. When p is less than or equal to 0.05, the effect of substrate type on growth rate is statistically significant. When p is a value between 0.05 and 0.10, the effect is considered marginally statistically significant. When p is greater than 0.10, the effect is not statistically significant. Table 1 was created using values from the SPSS statistical software tables, which can be found in the Appendix section. According to Figure 1, the leaf treatment showed the highest growth rate. The leaf treatment also showed a statistically significant difference when compared to the control, wood, and rock treatments. The wood treatment showed a statistically significant difference when compared to the rock treatment.

Table 1 P values from the pairwise contrasts of each treatment using univariate analysis of variance.

	Wood	Leaf	Rock
Control	0.072	**<0.001**	0.246
Wood		**<0.001**	**0.001**
Leaf			**<0.001**

Chapter 5

Discussion

The quality of food can be assessed by determining the growth rate of individuals and can influence invasive species (Dorgelo & Leonards, 2001). *P. antipodarum* feeds on different diets, including algae, bacteria, detritus, and fungi (Liess & Lange, 2011). When the New Zealand mud snail is in its native range, it can be found in high densities on pebble, cobble, and sand (Holomuzki & Biggs, 2007). On the other hand, when the snail is in an invaded range, it can be found in high densities on different substrates, including leaf litter, algae, wood, and gravel (Richards, et al., 2001) (Hall, et al., 2006) (Proctor, et al., 2007) (Davidson, et al., 2008). The snail feeds on the biofilms that grow on these substrates. Since each of these substrates foster different biofilms, they may have different effects on the growth rate of the snails.

The snail can also occupy different countries and continents, which shows that it has generalist traits that allow it to highly tolerate various habitats and environmental conditions (Geist, et al., 2022). *P. antipodarum* also shows high phenotypic plasticity due to its physical variation and life history traits, including its growth rate (Negovetic & Jokela, 2001). This study focuses on determining which substrate preferences result in the greatest growth rate for the invasive New Zealand mud snail.

A previous study shows that *P. antipodarum* has a higher growth rate when it consumes periphyton grown on rocks, in comparison to *Spirulina* algae powder, detritus, and detritus with periphyton grown on rocks (Bilka & Levri, 2013). The study mentions that it is possible that something in the detritus could have inhibited the growth or that the periphyton is considered a better food source for *P. antipodarum* (Bilka & Levri, 2013). Another study shows that the New Zealand mud snail prefers pebble and gravel over silt and sand (Lysne & Koetsier, 2006).

Accordingly, this study examines four different treatments. These treatments include periphyton grown on rocks, leaf litter, wood, and a control treatment containing *Spirulina* algae powder. The control treatment was used in another study, which found that a periphyton-based diet results in an increased growth rate compared to a detritus-based diet in *P. antipodarum* (Levri, et al., 2017). This demonstrates that periphyton is an appropriate food source for the snail. In the present experiment, the leaf treatment showed the highest mean growth rate. The wood treatment showed the second highest mean growth rate, followed by the control treatment. The rock treatment showed the lowest mean growth rate (Figure 1).

Periphyton on the rocks may have not been sufficient for the snails to grow at a faster rate than the other treatments, especially since the experiment was conducted during the fall in Pennsylvania, where sunlight might not have been adequate for optimal algal growth.

The wood treatment showed a higher mean growth rate than the rock treatment. This could be due to the high fungal growth in the plastic cups in the wood treatment since *P. antipodarum* feeds on fungi. Yet, the mean growth rate was not as high as the leaf treatments.

As for the leaf treatment, some of the plastic cups showed a lot of snail feces, while others did not. It appeared that the cups with snails with higher growth rates had more feces than the cups with the snails with lower growth rates.

This study demonstrates that a diet related to leaves and wood would likely result in higher growth rates of this invader. Therefore, we hypothesize that freshwater habitats with substantial inputs of woody and leafy debris may help to facilitate invasion. This, in turn, could increase the New Zealand mud snail’s invasion success.

An important limitation of this study is that the substrates utilized by the snails in this experiment were from the Spring Run stream and not from Spring Creek, where the snails originally came from.

Energy in aquatic systems can come from autochthonous sources, such as plants, and other photosynthetic organisms in the stream, such as algae. The energy can also come from organisms outside of the stream that wash in, like leaf litter and woody debris. This is known as allochthonous input. It appears that allochthonous energy input has a stronger effect on the growth of the mud snail. This means that streams that have more leaf litter and woody debris could be better habitats for the snail.

Chapter 6

Conclusion

In conclusion, the New Zealand mud snail shows different growth rates when it consumes food from different substrates, including periphyton grown on rocks, biofilms on wood, biofilms on leaf litter, and *Spirulina* algae powder. *P. antipodarum* shows the highest growth rate when it consumes leaf litter. The presence of feces is probably due to the snail feeding a lot when placed in this treatment. The second highest growth rate was with the wood treatment, which could be due to the presence of fungi. Fungi also decompose leaf litter. This is followed by the control treatment, which contained the *Spirulina* algae powder. The rock treatment showed the lowest growth rate, which could be a result of the insufficient algal growth on the rocks.

Consequently, the type of substrate that *P. antipodarum* consumes seems to influence the growth rate. This could promote the successful spread of the invasive species in different regions around the world, leading to decreased biodiversity, biotic homogenization, and altered habitat properties, such as fluctuating carbon and nitrogen cycles in invaded ecosystems.

Chapter 7

Appendix

Table 2 Results from a univariate analysis of variance comparing log transformed data of all treatments.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	18.192[a]	3	6.064	37.237	<0.001
Intercept	195.044	1	195.044	1197.699	<0.001
Treatment	18.192	3	6.064	37.237	<0.001
Error	9.445	58	0.163		
Total	226.274	62			
Corrected Total	27.637	61			

Table 3 Results from a univariate analysis of variance comparing log transformed data of the control treatment to the leaf treatment.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	11.145[a]	1	11.145	60.295	<0.001
Intercept	65.387	1	65.387	353.758	<0.001
Treatment	11.145	1	11.145	60.295	<0.001
Error	4.991	27	0.185		
Total	79.750	29			
Corrected Total	16.135	28			

Table 4 Results from a univariate analysis of variance comparing log transformed data of the control treatment to the rock treatment.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.217[a]	1	0.217	1.401	0.246
Intercept	149.570	1	149.570	963.678	<0.001
Treatment	0.217	1	0.217	1.401	0.246
Error	4.501	29	0.155		
Total	156.819	31			
Corrected Total	4.718	30			

Table 5 Results from a univariate analysis of variance comparing log transformed data of the control treatment to the wood treatment.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.697[a]	1	0.697	3.488	0.072
Intercept	115.909	1	115.909	580.016	<0.001
Treatment	0.697	1	0.697	3.488	0.072
Error	5.595	28	0.200		
Total	121.518	30			
Corrected Total	6.292	29			

Table 6 Results from a univariate analysis of variance comparing log transformed data of the leaf treatment to the rock treatment.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	15.817[a]	1	15.817	123.260	<0.001
Intercept	80.241	1	80.241	625.289	<0.001
Treatment	15.817	1	15.817	123.260	<0.001
Error	3.850	30	0.128		
Total	104.756	32			
Corrected Total	19.667	31			

Table 7 Results from a univariate analysis of variance comparing log transformed data of the leaf treatment to the wood treatment.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6.769[a]	1	6.769	39.702	<0.001
Intercept	56.414	1	56.414	330.893	<0.001
Treatment	6.769	1	6.769	39.702	<0.001
Error	4.944	29	0.170		
Total	69.455	31			
Corrected Total	11.713	30			

Table 8 Results from a univariate analysis of variance comparing log transformed data of the rock treatment to the wood treatment.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.851[a]	1	1.851	12.878	0.001
Intercept	139.116	1	139.116	968.107	<0.001
Treatment	1.851	1	1.851	12.878	0.001
Error	4.455	31	0.144		
Total	146.524	33			
Corrected Total	6.305	32			

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ACADEMIC VITA

SALLY GHANNAM

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Education

Pennsylvania State University — Graduated May 2022
Biology (Vertebrate Physiology Option), Bachelor of Science — **Dean's List** Fall 2018 – Spring 2022

Research Experience

- Undergraduate Research | Dr. Edward Levri | Pennsylvania State University — January 2021 – May 2022
  Performed field research, maintained snails in the laboratory, and conducted research to determine the optimal substrate for the invasive New Zealand mud snail (*Potamopyrgus antipodarum*).
- Undergraduate Research | Dr. Todd Cook | Pennsylvania State University — August 2019 – May 2020
  Conducted a comparative analysis of shark species' dental serration morphology and histology. The research focused on determining if there are differences in tooth histology between species of *Squalicorax* and if the tissues that make up the serrations are similar to those of *Galeocerdo cuvier*.

Professional Experience

Job Shadowing

- UPMC Altoona Hospital (inpatient, palliative care, pediatrics, and OB), Altoona Family Physicians (AFP), Women's Health and Wellness (WHW), Pregnancy Care Center (PCC), Musculoskeletal Clinic, Endocrine Clinic, and Osteopathic Manipulative Treatment (OMT) Clinic | Altoona, Pennsylvania — August 2021 – December 2021
- Dental Clinic, Craig K. Mathias, D.D.S., M.S., Preceptor | Harrisburg, Pennsylvania — May 2019
- Dental Clinic, ZahnArts: Joseph Massis, D.D.S., Perceptor | Nordhorn, Germany — June 2017 – July 2017

Professional Organizations & Involvement

- Penn State Pre-Dental Society Member | Pennsylvania State University — August 2021 – May 2022
- Tri Beta National Biological Honor Society and Biology Club Member | Pennsylvania State University — September 2020 – May 2022
- Scrubs Club Member | Pennsylvania State University — February 2019 – May 2020
- Global Boarders Program Member | Pennsylvania State University — January 2019 – May 2020

Community Service

- Spring Run Stream Clean-up | Altoona, Pennsylvania — September 2021
- Pennsylvania Soldiers' and Sailors' Home | Erie, Pennsylvania — February 2019 – April 2020
- Schriner's Hospital for Children in Erie | Erie, Pennsylvania — January 2020 – April 2020
- Linked by Pink | Erie, Pennsylvania — October 2019
- Adopt-A-Highway | Erie, Pennsylvania — October 2019
- Anna Shelter | Erie, Pennsylvania — March 2019

Leadership Experience

- Judge at the 2022 Region 10 Virtual PJAS Competition | Pennsylvania Junior Academy of Science — February 2022
- Tutor for Biology, Chemistry, Algebra, Calculus I and Calculus II, German, Writing, Communication Arts and Sciences, Introductory Microeconomics, and Introductory Macroeconomics | Pennsylvania State University — August 2019 – May 2020
- Global Ambassador | Pennsylvania State University — May 2019 – May 2020
- Organization of Latin American Students Vice President | Pennsylvania State University — April 2019 – May 2020
- Lambda Sigma National Honor Society President | Pennsylvania State University — March 2019 – May 2020
- Teaching Assistant for German | Pennsylvania State University — August 2019 – December 2019
- Behrend Honors Student Association Secretary | Pennsylvania State University — March 2019 – December 2019

Honors & Awards

- Penn State University Libraries Undergraduate Research Award — April 2022
- Kristina K. Peachman Undergraduate Student Research Award for Science | Pennsylvania State University — March 2022 – May 2022
- Veronesi-Iffert Open Door Scholarship | Pennsylvania State University — February 2022 – May 2022
- Bechtel-Wherry Open Doors Scholarship | Pennsylvania State University — February 2022 – May 2022
- Colfelt Scholarship | Pennsylvania State University — February 2022 – May 2022
- Honors Program Award | Pennsylvania State University — January 2022 – May 2022
- Class of 1922 Memorial Scholarship | Pennsylvania State University — August 2021 – May 2022
- Altoona Internal Grant | Pennsylvania State University — August 2021 – May 2022
- Schreyer Honors College | Pennsylvania State University — August 2020 – May 2022
- Altoona Honors Program | Pennsylvania State University — August 2020 – May 2022
- S & T Ross Trustee Scholarship | Pennsylvania State University — August 2020 – May 2022
- Penn State Behrend Academic Excellence Award | Pennsylvania State University — August 2019 – May 2020
- Phillips Scholarship | Pennsylvania State University — January 2019 – May 2020
- Behrend Honors Program | Pennsylvania State University — January 2019 – May 2020
- Early Acceptance Program for Dental School | Lake Erie College of Osteopathic Medicine — September 2019
- Behrend Faculty and Staff Scholarship | Pennsylvania State University — May 2019 – August 2019

Languages

- Fluent in English
- Fluent in Arabic
- Intermediate in Spanish
- Intermediate in German